Chloral Hydrate Metabolites by Headspace GC/MS(EI)

1 Introduction

Chloral hydrate is a sedative-hypnotic agent that is rapidly metabolized to trichloroethanol (TCE) which is excreted unchanged and as a glucuronide conjugate. TCE is further metabolized to trichloroacetic acid (TCA), which is also excreted in the urine. TCA has a very long elimination half-life, and can be detected in the urine days after chloral hydrate consumption. Chloral hydrate is infrequently prescribed, but may be used in a drug-facilitated assault.

2 Scope

This procedure allows for the screening and confirmation of TCE and TCA in urine. This document applies to Chemistry Unit case working personnel who perform toxicology analyses.

3 Principle

Urine is mixed in a headspace vial with sulfuric acid and dimethyl sulfate. The dimethyl sulfate is used to form the methyl ester derivative of TCA. The sulfuric acid cleaves any glucuronide conjugated TCE and aids in the derivatization of TCA. Vials are heated at 60°C for three hours, and the headspace is analyzed by GC/MS(EI).

4 Specimens

This procedure uses 1 mL of urine. Smaller volumes may be used, with appropriate dilution, if available specimen is limited.

5 Equipment/Materials/Reagents

a. Agilent Gas Chromatograph/Mass Spectrometer equipped with a headspace autosampler and a 30 m x 0.25 mm x 1.4 µm DB-624 column, or equivalent

b. 20-mL disposable headspace vials, magnetic caps, and crimper

c. Volumetric flasks (10 and 100 mL)

d. Pipetters and disposable tips

e. Sulfuric Acid (Reagent grade, or better)

f. Dimethyl Sulfate (99.8% pure)

g. Disposable syringes with hypodermic needles

6 Standards and Controls

a. 2,2,2-Trichloroethanol (99+%)

b. Trichloroacetic acid (Sigma Ultra, 99.0%)

c. TCE Working Standard Solution (1.0 mg/mL):
Add 65 µL trichloroethanol to about 90 mL deionized water in a 100-mL volumetric flask. Dilute to the mark with deionized water and mix thoroughly. Store refrigerated in a tightly sealed glass or plastic container. Stable for 2 months.

d. TCA Working Standard Solution (1.0 mg/mL):
Add 10 mg trichloroacetic acid to a 10-mL volumetric flask. Dilute to the mark with deionized water and mix thoroughly. Store refrigerated in a tightly sealed glass or plastic container. Stable for 2 months.

e. Negative Control Urine:
Obtain in house. Store refrigerated in plastic. Stable for at least one year. A Negative Control Urine sample will be analyzed with every assay.

f. Positive Control Urine:
To 1 mL aliquots of urine, add 20 µL of the TCE and TCA Working Standard Solutions to prepare a 20 µg/mL Positive Control Sample, or 100 µL of each Working Standard Solution to prepare a 100 µg/mL Positive Control Sample. At least one Positive Control Urine sample will be analyzed with every assay.

7 Sampling

. Representative portions of the specimens are obtained. See TOX101 for further details.

8 Procedure

Appendix 1 contains an abbreviated version of this procedure. This form may be used at the bench by the authorized individual performing the procedure.

a. Into properly labeled 20-mL headspace vials add 1 mL of urine. Positive controls may be prepared directly in the headspace vial.

b. Immediately cap.

c. Add 0.5 mL concentrated sulfuric acid through the cap using a disposable syringe with a hypodermic needle and vortex.

d. Add 0.1 mL dimethyl sulfate through the cap using a disposable syringe with a hypodermic needle and vortex.

e. Analyze specimens by headspace GC/MS(EI) after confirming that the instrument is calibrated and in proper working condition.

9 Instrumental Conditions

9.1 Headspace Sampler Parameters

incubation temperature	60°C	syringe temperature	90°C
incubation time	180 min	sample fill volume	2.5 mL
agitator speed	300 RPM	sample fill rate	1.0 mL/sec
agitation timing	10 sec on 1 sec off	sample fill strokes	5
injection volume	1.0 mL	sample injection speed	1.0 mL/sec
		syringe flush time	240 sec

9.2 Gas Chromatograph Parameters

Oven Parameters		Column Parameters		Inlet and Carrier Parameters	
temperature 1	50°C	type	DB-624	inlet temp.	150°C
hold 1	3 min	length	30 m	injection mode	split
ramp 1	10°C/min	internal diameter	0.25 mm	carrier gas	ultrapure helium
temperature 2	250°C	film thickness	1.4 µm	carrier mode	constant pressure
hold 2	15 min			pressure	6.54 psi
total run time	38 min			split ratio	10:1

9.3 Mass Spectrometer Parameters

ionization mode	electron impact (+)	source temperature	230°C
scan mode	Scan/SIM	transfer line temperature	260°C
scan range	29 - 200 m/z	quadrupole temperature	150°C
multiplier offset	+106 V	solvent delay	4 min (approximate)
SIM group 1 (4-12 min)	59, 82, 117, 119	dwell times	20
SIM group 2 (12-38 min)	31, 49, 77, 113		

10 Decision Criteria

The criteria in sections 10.1 through 10.2 are used as guidelines in determining the acceptability of the data produced in this assay. In general, compound identification should be based on a comparison of the chromatography and mass spectrometry for the analyte peak of interest with data from a contemporaneously analyzed reference standard, calibrator, or positive controls.

10.1 Chromatography

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. In order to be determined acceptable, a chromatographic peak in an unknown sample should compare favorably to a chromatographic peak of the same analyte in a known sample analyzed on the same system in the same or subsequent analytical runs. Additionally, the following two criteria should be met.

10.1.1 Retention Time

The retention time of the peak should be within ±2% of the retention time (relative or absolute, as appropriate) obtained from injection of a reference standard, or an extracted positive control. The relative retention times of the components should agree with those listed in the enclosed table within ±2%. If not, the shift in relative retention times should be noted and appropriate corrections made when analyzing the data generated from case specimens.

Expected Retention Times for TCA-methyl ester and TCE

Analyte	Expected RT (min)
TCA-methyl ester	11.843
TCE	12.239

10.1.2 Signal-to-Noise

To justify the existence of a peak, its signal-to-noise ratio should exceed 3. Further, the baseline signal for the peak of interest should be at least 10-fold greater than that for any observed peak at similar retention time in a Negative Control or blank injected just prior to the sample.

10.2 Mass Spectrometry

The mass spectrum of the analyte of interest should reasonably match that of a reference standard, calibrator, or Positive Control. See the *Guidelines for Comparison of Mass Spectra* (TOX104) technical procedure for further guidance.

11 Calculations

Not applicable.

12 Measurement Uncertainty

Not applicable.

13 Limitations

a. Limits of Detection:
1 µg/mL for TCE
0.5 µg/mL for TCA

b. Interferences:
None known.

14 Safety

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

15 References

Breimer, D.D., Ketelaars, H.C.J. and Van Rossum, J.M. "Gas Chromatographic Determination of Chloral Hydrate, Trichloroethanol and Trichloroacetic Acid in Blood and Urine Employing Headspace Analysis", *J Chrom*, 1974, *88*, 55-63.

Rev.#	Issue Date	History	
1	08/23/2012	Updated chromatography decision criteria in Section 11.1. Added expected retention times to Section 11.1.1.	
2	09/01/2021	Entire document	Removed footers.
		Document	Updated approval lines.
		1	Clarified that chloral hydrate is a sedative-hypnotic.
		2	Updated scope to current standard language.
		3	Clarified language
		4	Added possibility of specimen dilution.
		5	Added volumetric flasks and removed unnecessary brand names. Updated manufacturer name.
		(7)	Removed section (calibration) and renumbered subsequent sections. Added sampling language.
		8 and Appendix 1	Changed “examiner or chemist” to “authorized individual” in preamble. Added instruction to vortex samples after reagent addition.
		9.3 and Appendix 1	Changed amu to m/z, added “approximate” for solvent delay
		10.1.2	Removed requirement of specific signal-to-noise algorithm.
		10.2	Removed “reasonable degree of scientific certainty” language
		12	Changed title to “Measurement Uncertainty”
		15	Removed references to FBI Laboratory internal documents.
		6, 13	Changed PPM to µg/mL throughout.
		Appendix 1	Added headspace injection volume.

Approval

Redact - Signatures on File

Chemistry Unit Chief: Date: 08/31/2021

Toxicology Technical Leader: Date: 08/31/2021

Appendix 1: Abbreviated version of the Chloral Hydrate Metabolite Procedure for bench use.

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